

CLAIMS

1. A culture medium, characterized in that it comprises chicken serum and a culture support
5 comprising inactivated 3T3 cells of the cell line registered in the ATCC collection under the number CCL92.

2. The culture medium as claimed in claim 1,
10 characterized in that the proportion by weight of chicken serum relative to the total weight of the culture medium is between 0.5 and 50%.

3. The culture medium as claimed in either one of the
15 preceding claims, characterized in that the 3T3 cells are derived from the 3T3 cells of the cell line registered in the ATCC collection under the number CCL92 by means of a method containing the steps consisting of inoculation into wells, culture,
20 transfer, culture, detachment by trypsinization, freezing half of each subclone, treatment of the other half with mitomycin C or with gamma rays, incubation of chicken keratinocytes on each treated subclone, culture, fixing and staining with rhodamine B, and
25 selection of the subclone on which there are the largest number of keratinocyte colonies of the largest size.

4. The culture medium as claimed in any one of the
30 preceding claims, characterized in that the 3T3 cells have been inactivated by an antimitotic treatment selected from treatment with mitomycin C or irradiation with gamma rays.

35 5. The culture medium as claimed in any one of the preceding claims, characterized in that it also contains one or more of the following constituents: inorganic salts, vitamins, hormones and growth factors.

6. The culture medium as claimed in any one of the preceding claims, characterized in that it also contains one or more of the following constituents:
- 5 inorganic salts, amino acids, vitamins, fatty acids, glucose, a buffer, phenol red, EGF (epidermal growth factor), T₃, hydrocortisone, insulin, cholera toxin, transferrin and adenine.
- 10 7. A keratinocyte culture, characterized in that it contains a culture medium that comprises chicken serum and a culture support comprising inactivated mammalian cells, preferably inactivated fibroblasts.
- 15 8. The keratinocyte culture as claimed in claim 7, characterized in that it contains a culture medium as claimed in any one of the preceding claims 1 to 6.
9. The keratinocyte culture as claimed in claim 8,
- 20 characterized in that the keratinocytes are avian keratinocytes.
10. The keratinocyte culture as claimed in claim 9,
- 25 characterized in that the keratinocytes are chicken keratinocytes.
11. A method of culturing epidermal cells or keratinocytes, said method comprising the steps consisting in:
- 30 (i) preparing a suspension of keratinocytes;
- (ii) inoculating said cells into a culture medium comprising chicken serum and a culture support comprising inactivated fibroblasts;
- 35 (iii) incubating said cells in this culture medium.
12. The method as claimed in claim 11, characterized in that the culture medium is as defined in one of claims 1 to 6.

13. The method of culture as claimed in claim 11 or claim 12, characterized in that, at the end of step (iii), the cells derived from the culture are recovered
5 and a further culture cycle is applied to them according to steps (ii) and (iii) of the method as claimed in claim 11.

14. The method of culture as claimed in claim 13,
10 characterized in that it comprises at most 30 cell culture cycles.

15. A method of propagating a virus *in vitro*, said method comprising the steps consisting in:

- 15
- (i) infecting keratinocytes with the strain of the virus;
 - (ii) placing the infected keratinocytes in culture according to the method of claims 11 to 14;
 - 20 (iii) extracting the virions produced in step (ii).

16. The method as claimed in claim 15, characterized in that it also comprises a step consisting of lyophilization of the virions.

25

17. The method as claimed in claim 15 or claim 16, characterized in that the keratinocytes are chicken epidermal cells.

30

18. A complete, acellular, live, attenuated enveloped DNA virion obtained from a virus chosen from MDV and FPV, characterized in that it can be obtained by means of a method as claimed in any one of claims 15 to 17.

35

19. A live attenuated vaccine, characterized in that it contains at least one virion as claimed in claim 18 and a vaccine support.

20. The vaccine as claimed in claim 19, characterized
in that it is in the form of a kit, comprising,
firstly, the lyophilized virion and, secondly, the
immunization support, each one being packaged in a
5 separate compartment.

21. The vaccine as claimed in claim 19 or claim 20,
characterized in that it is intended for administration
by injection, oral administration, administration via
10 the *in ovo* route or administration via the respiratory
pathway.

22. The use of a virion as claimed in claim 18, for
preparing a medicinal product for use in the prevention
15 or treatment of Marek's disease.